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| EXAMINER | |
|---|---|
| SGAGIAS, MAGDALENE K | |
| ART UNIT | PAPER NUMBER |
| 1632 | |
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/855,587 | SASAI ET AL. | |
| | Examiner | Art Unit | |
| | MAGDALENE SGAGIAS | 1632 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *20 October 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1,18-21,23,24,74,75 and 80-90* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,18-21,23,24,74,75 and 80-90* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *29 January 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

**DETAILED ACTION**

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 12/07/2010 has been entered.

Claims 1, 18-21, 23-24, 74-75, 80-90 are pending and under consideration. The amendment dated 12/07/2010 has been entered. Claims 2-17, 22, 25-73, 76-79 are canceled.

The declaration of Dr. Yoshiki Sasai dated 12/07/2010 has been considered.

***Claim Rejections - 35 USC § 112/Necessitated by Amendment***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The rejection of claims 1, 18-21, 23-24, 74-75, 80-90 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is withdrawn in view of the declaration dated 12/07/2010 for culturing the claimed embryonic stem cells in the absence of retinoic acid.

Applicant's arguments are convincing regarding culturing the embryonic stem cells in the absence of retinoic acid.

The rejection of claims 1, 18-21, 23-24, 74-75, 80-90 under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for culturing a mouse embryonic

stem cell in vitro in the presence of a stroma cell without forming embryoid body, wherein the stroma cell OP9 cell or PA6 cell, does not reasonably provide enablement for culturing a human embryonic stem cell in vitro in the presence of a stroma cell without forming embryoid body, wherein the stroma cell OP9 cell or PA6 cell, and wherein the embryonic stem cell is selected from the group consisting of (b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of he nucleus of a somatic cell; and (c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (b) is modified using gene engineering is withdrawn in view of the amendment dated 12/07/2010.

Claims **1, 18-21, 23-24, 74-75, 80-90** are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for culturing mouse embryonic stem cells with serum free medium in the absence of retinoic acid and in the presence of a stroma cells without forming embryoid body, wherein the stroma cell is OP9 cell or PA6 cell to produce a neural cell expressing a neural crest marker or a neural tube marker, does not reasonably provide enablement for producing a neural cell expressing a neural crest marker or a neural tube marker, from various embryonic stem cells or inducing differentiation of various embryonic stem cells derived form different organisms into a cell expressing a neural crest marker or a neural tube marker, by culturing the embryonic stem cells with serum free medium in the absence of retinoic acid and in the presence of a stroma cells without forming embryoid body, wherein the stroma cell is OP9 cell or PA6 cell. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The claims are directed to a method for producing a cell expressing a neural crest marker or a neural tube marker, comprising: culturing under serum-free conditions an embryonic

stem cell in vitro in the absence of retinoic acid and in the presence of a stroma cell without forming embryoid body, wherein the stroma cell is OP9 cell or PA6 cell. Claims 24 and 74 specify the method according to any one of claims 1, 80 or 81, wherein the embryonic stem cell is selected from the group consisting of: (a) an embryonic stem cell established by culturing an early embryo before implantation; (b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and (c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering. Independent claim 80 is directed to a method for producing a dopaminergic neuron, an acetylcholinergic neuron, a 7-aminobutyratergic neuron or a serotonergic neuron, comprising:

culturing under serum-free conditions an embryonic stem cell in vitro in the absence of retinoic acid and in the presence of a stroma cell without forming embryoid body, wherein the stroma cell is OP9 cell or PA6 cell. Independent claim 81 is directed a method for producing a neural stem cell which is stained by an anti-nestin antibody comprising: culturing under serum-free conditions an embryonic stern cell in vitro in the absence of retinoic acid and in the presence of a stroma cell without forming embryoid body, wherein the stroma cell is OP9 cell or PA6 cell.

The claims encompass producing neural cells from various embryonic stem cells or inducing differentiation of various embryonic stem cells derived from numerous different organisms, such as humans, monkeys, chimpanzees, other primates, mice, rats, cows, horses, pigs, sheep, whales, other mammals, insects, birds, and fishes etc., into neurons by culturing under serum-free conditions an embryonic stem cell in vitro in the absence of retinoic acid and in the presence of a stroma cell without forming embryoid body, wherein the stroma cell is OP9 cell or PA6 cell. The specification discloses growing EB5 ES cells (mouse ES cells) in serum-free medium with a stroma cell, MC3T3-G2/PA6 cell and demonstrates that after 8 days induces

differentiation of the mouse embryonic stem cells into neural cells expressing crest marker AP-2 or neural tube marker NKk2.2 and Pax-7 (examples 1 and 14). However, the specification fails to provide adequate guidance and evidence for how to produce neural cells from various embryonic stem (ES) cells derived from numerous organisms by culturing the ES cells without forming embryoid body under serum free conditions.

Cell markers for different stem cells differ from each other dramatically because they differ biochemically and physiologically. Stem cells from different organism also differ from each other dramatically. For example, there are dramatic molecular and cellular differences between human and mouse embryonic stem cells. **Allegrucci et al., 2006** (Human Reproduction Update, Vol. Advance Access published on August 26, 2006, p. 1-18) demonstrates that there is difference in pluripotency marker molecules, transcriptional profiling, genetic stability and epigenetic stability even among different human embryonic stem cell lines (e.g. abstract). There are differential expression of different markers among different human ES cell lines and "[t]he physiological significance of expression of these markers is not clear, and it is likely that the limited panel of markers in current use may be insufficient to define the state of 'stemness' because many of them are not unique to embryonic stem cells" (e.g. p. 2, right column). **Sato et al., 2003** (Developmental Biology, Vol. 260, p. 404-413) shows that there are 918 different gene expressions between human embryonic stem cell line H1 (HESC H1 line) and mouse embryonic stem cells (MESCs) (e.g. Figure 4A). There are molecular markers that are unique for HESCs as compared to MESCs, for example, SOCS-1, an inhibitor of the STAT-3 signaling pathway, is enriched in HESCs but not in MESCs (e.g. p. 412, left column, 3rd paragraph). Sato also suggests that different human ES lines have different transcriptional profiles and respond differently to the differentiation conditions (e.g. p. 412, left column, last paragraph). **Rao, M., 2004** (Developmental Biology, Vol. 275, p. 269-286) reports some known differences between

mice and human ES cells (e.g. table 3). The difference between mice and human ES cells is much higher than that seen in human-to-human cell comparison (e.g. p. 282, left column, 1st paragraph).

Indeed, there are different molecular markers even among different human ES cells. **Abeyta et al., 2004** (Human Molecular Genetics, Vol. 13, No. 6, p. 601-608) compares gene expression profiles of different human ES cell lines, HSF-1, HSF-6 and H9 lines. Abeyta observed that each line has a unique expression signature and the expression of many genes was limited to just one or two hESC lines. Abeyta suggests that "the observed differences in gene expression between independently-derived hESC lines may reflect inherent differences in the initial culture of each line and/or the underlying genetics of the embryos from which the lines were derived" (e.g. abstract). It appears that human, rat and mouse ES cells could have different specific cell markers (human and mice have dramatically different expression profiles) and the cell markers could be different even among different human ES cells, and there are differential expressions even among common cell markers. Differences in gene expression between independently-derived hESC lines may reflect inherent differences in the initial culture of each line and/or the underlying genetics of the embryos from which the lines were derived. The mechanisms of differentiation from various ES cells to nerve cells also differ from each other. **Sasai Y.,** 2002 (Journal of Neurology, Vol. 249, Supplement 2, p. II/41-II/44) points out difference between neural induction in Xenopus and mouse. Chordin, noggin and follistatin are known neural inducer in Xenopus, however, introduction of pCMV-chordin plasmid into ES cells or addition of follistatin protein to culture medium does not induce significant neural differentiation of mouse ES cells. Sasai suggests that "mouse ES cells, unlike Xenopus cells, require presently unknown signals for the initiation of neural differentiation, in addition to the attenuation of BMP signals" (e.g. p. II/42, bridging left and right column). It appears that ES

cells derived from different organisms could have diverse mechanisms of differentiation into nerve cells and require different factors to reach differentiation into neurons. Diverse factors determine the differentiation of various ES cells into nerve cells and the type of medium condition used also could affect the differentiation from ES cells to nerve cells.

A search for the state of the art of differentiation from ES cells to neural cells shows that no report has been published regarding non-embryoid body formation to induce differentiation of ES cells into neural cells at the time of the filing of the instant invention as instantly claimed other than mouse. There is no evidence of record that ES cells derived from numerous organisms other than mouse would be able to differentiate into neural cells by using co-culture with OP6 or PA6 feeder cells under non-aggregation condition with serum-free medium. Absent specific guidance, one skilled in the art at the time of the invention would not know how to differentiate the ES cells other than mouse ES cells into nerve cells by using the claimed method. The specification only discloses differentiation of mouse ES cells into neural cells under where no EB formed and under serum-free medium. Absent specific guidance, one skilled in the art at the time of the invention would not know how to practice over the full scope of the invention claimed.

For the reasons set forth above, one skilled in the art at the time of the invention would require undue experimentation to practice over the full scope of the invention claimed. This is particularly true based upon the nature of the claimed invention, the state of the art, the unpredictability found in the art, the teaching and working examples provided, the level of skill which is high, the amount of experimentation required, and the breadth of the claims.

As a second issue, claims 24 and 74 specify the method according to any one of claims 1, 80 or 81, wherein the embryonic stem cell is selected from the group consisting of: (a) an embryonic stem cell established by culturing an early embryo before implantation; (b) an

embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and (c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

The specification teaches co-culture of PA6 cells with the mouse EB5 cells in serum free medium and comparing the affect of the presence and absence of BMP4 in the serum free medium during the culturing/differentiation of the mouse ES cells (example 14). The specification teaches the co-culture of PA6 cells with the mouse EB5 cells in serum free medium and after 8 days stained for the nestin neural specific marker and after 10 days stained for dopaminergic marker, cholinergic neuron marker, GABAnergic marker serotonergic a marker or dopaminergic marker (specification p. 93, example 1). However, the specification has failed to provide guidance for a method according to any one of claims 1, 80 or 81, wherein the embryonic stem cell is selected from the group consisting of: (a) an embryonic stem cell established by culturing an early embryo before implantation; (b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and (c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering as instantly claimed.

The art teaches that it is unpredictable to (a) having a human embryonic stem cell by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell and (b) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell (a) is modified using gene engineering. Although one might be able to isolate a particular cell type from an NT-produced mouse embryo, a NT-produced human embryo and a bioengineered human embryonic stem from a NT-produced human embryo is not found to be predictable. The instant invention is not enabling because the claims as written require that a human embryonic stem cell is derived from a human embryo produced nuclear transplantation, be able to

differentiate into a neural cell in vitro, the state of the art of which is unpredictable. For example, **Lerou et al,** (Blood Reviews, 19: 321–331, 2005 (IDS)) teaches theoretically, while generating ESC from somatic nuclear cell transfer embryos in mice is well established in 2004, a group of scientists from Korea reported deriving the first hESC line from a human blastocyst created using somatic cell nuclear transfer however, it remains to be seen whether mitochondrial DNA which would be oocyte derived (p 325, 1st column, 1st paragraph). Thus, the unpredictability in the claimed invention is to use these cells to engineer differentiated human ES cells into neural cells in vitro. The claims require the engineering of cells isolated from the embryo produced by nuclear transfer. However, as noted above, the state of the art teaches that the production of a NT-produced human embryo, as required by the claims, for the culture of human embryonic stem cells to differentiate into neural cells as instantly claimed would be unpredictable. The instant specification fails to provide teachings or guidance to address or overcome the above-noted unpredictability's that the state of the art teaches with regard to the generation of a NT-produced human embryo resulting in human embryonic stem to be cultured and differentiated into neural cells in vitro. As such, the instant specification fails to enable the invention.

The instant specification does not provide any relevant teachings, specific guidance, or working examples for overcoming the limitations of generation of a NT-produced human embryo resulting in human embryonic stem to be cultured and differentiated into neural cells in vitro raised by the state of the art. The instant specification does not provide guidance for growth factor conditions requirement needed for human ES cells not applied to mouse ES cells.

Therefore, the skilled artisan would conclude that the state of art of generation of a NT-produced human embryo resulting in human embryonic stem to be cultured and differentiated into neural cells in vitro by way of the claimed method is undeveloped and unpredictable at best. Given the lack of guidance provided by the instant specification, it would have required undue

experimentation to practice the invention as claimed for generation of a NT-produced human embryo resulting in human embryonic stem to be cultured and differentiated into neural cells in vitro without a reasonable expectation of success.

Therefore, in view of the quantity of experimentation necessary to determine the parameters listed above for the for generation of a NT-produced human embryo resulting in human embryonic stem to be cultured and differentiated into neural cells in vitro, the lack of direction or guidance provided by the specification for generation of a NT-produced human embryo resulting in human embryonic stem to be cultured and differentiated into neural cells in vitro, the absence of working examples that correlate to generation of a NT-produced human embryo resulting in human embryonic stem to be cultured and differentiated into neural cells in vitro, the undeveloped state of the art pertaining to generation of a NT-produced human embryo resulting in human embryonic stem to be cultured and differentiated into neural cells in vitro, and the breadth of the claims directed to all types of bioengineered human embryonic stem cells from NT-produced human embryo, it would have required undue experimentation for one skilled in the art to make and/or use the claimed invention. Therefore, the skilled artisan would conclude that the state of art of producing neural cells as instantly claimed is undeveloped and unpredictable at best. Given the lack of guidance provided by the instant specification, it would have required undue experimentation to practice the invention as claimed for producing these cells without a reasonable expectation of success.

A Applicants argue the submitted PNAS, Vol. 99 (2002) 1580 and pointed out that even in monkey ES cells, differentiation of dopaminergic neuron is induced by the method of the present invention. If the Examiner is now aware of information why such showing is irrelevant, she is respectfully requested to provide a suitable affidavit under MPEP §2144.03. Applicant's arguments have been fully considered but are not persuasive.

In response, the above PNAS paper specifically describes that primate ES cells were cultured in serum-free medium on feeder cells **but not** without forming embryoid body (see p 158-, 32nd column under maintenance of primate ES cells and p 181, 1st column, 1st paragraph). Thus, the PNAS paper is not within the scope of the claims because it does not discuss differentiating ES cells without forming an embryoid body. Therefore, Applicants have not provided guidance to overcome the unpredictability of culturing primate ES cells under conditions as instantly claimed.

B. Applicants argue to the extent the Examiner may be no longer persuaded by this argument, Cell, Vol. 131, No. 5 (2007) 861-72 (copy attached) shows the method of the present invention can be applied to human ES cells. See the first sentence in right column on page 864 ("human iPS cells could be induced by reported methods for hES cells"). Applicant's arguments have been fully considered but are not persuasive.

In response, the above cited Cell paper and specifically the first sentence in right column on page 864 is directed to differentiation of human iPS cells into neurons and not into differentiation of ES cells as instantly claimed. First, it is well known the art of iPS cell technology that the generation of murine iPS cells from fibroblasts by expression of transcription factors was first reported in 2006. The Cell paper published in 2007 is a post filing art and describes the generation of embryoid body-mediated differentiation of human iPS cells. That is, the Cell paper is not within the scope of the claims, which are directed to a method of culturing an embryonic stem cell without forming embryoid body. Thus, the Cell paper is a post filing art which does not enable the instant claims at the time of filling. Second, the instant claims require an embryonic stem cell while the iPS cells are similar to ES cells in morphology, proliferation, ability to differentiate along a given lineage and express cell surface markers that characterize ES cells; however, as of today it is not clear how iPS cells are similar to ES cells. Third, while

the PNAS paper discusses culturing of monkey ES cells and the Cell paper discusses human iPS cells it is well known in the art that culturing conditions of monkey ES cells are distinct from culturing iPS human cells. Therefore, Applicant's have not provided guidance to overcome the unpredictability of culturing ES cells under conditions as instantly claimed.

***Claim Rejections - 35 USC § 103/Necessitated by Amendment***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The rejection of claims 1, 23, 81, 82-87 under 35 U.S.C. 103(a) as being unpatentable over **Nakano et al,** (Science, 265: 1090-1101, 1994) in view of **Samarut et al** (US 6,114,168) is withdrawn in view of the amendment dated 12/07/2010.

Applicant's arguments are convincing.

***Conclusion***

**No claim is allowed.**

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Magdalene K. Sgagias whose telephone number is (571)272-3305. The examiner can normally be reached on Monday through Friday from 9 AM to 5:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Paras Peter can be reached on 571-272-4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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